

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
Group Art Unit 1637

In re Patent Application of

Electronically filed by Sally Sorensen on June 25, 2010

Andrei Laikhter, et al.

Application No. 10/666,998

Confirmation No.: 1003

Filed: September 19, 2003

Examiner: Mark Staples

"ANTHRAQUINONE QUENCHER DYES,  
THEIR METHODS OF PREPARATION  
AND USE"

**DECLARATION OF MARK BEHLKE UNDER 37 CFR § 1.131**

Mail Stop Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

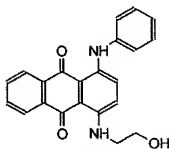
I, Mark Behlke, do hereby declare and state the following:

1. I am an inventor of one or more claims of the above-identified application, along with Dr. Andrei Laikhter, Dr. Yawfui Yong, Dr. Scott Rose, and Dr. Lingyan Huang.
2. I have read and understand the invention as disclosed in the present application, as defined by the presently pending claims.
3. I understand that a Request for Reconsideration was filed in order to obtain consideration of an abstract entitled "A Novel Dark Quencher for Oligonucleotide Probes: Synthesis and Applications", authored by J.P. May et al. and distributed to attendees of the May 6-8 2002 Oligonucleotides ("TIDES") Technology Conference on May 6, 2002, was submitted to the United States Patent and Trademark Office in an Information Disclosure Statement on October 23, 2003, which the Office mistakenly failed to consider previously.
4. On April 12, 2010, I executed a Declaration Under 37 CFR § 1.131 ("the April 12, 2010 Declaration") providing evidence that, prior to May 6, 2002 (a) Dr. Laikhter synthesized mono-

and di-  $\alpha$ -aminoanthraquinone quencher phosphoramidite monomers, precursors for attaching the quenchers to oligonucleotides (See Exhibit A of the April 12, 2010 Declaration), and (b) the oligonucleotides labeled with a fluorophore and with the  $\alpha$ -aminoanthraquinone quenchers synthesized by Dr. Laikhter were used to detect a target nucleic acid sequence in a sample, according to the claimed methods (See Exhibit B of the April 12, 2010 Declaration). I understand that the April 12, 2010 Declaration was filed with the United States Patent and Trademark Office (USPTO) on April 13, 2010.

5. I understand that the USPTO has requested supplemental information explaining how Exhibit B of the April 12, 2010 Declaration describes oligonucleotide probes labeled with a fluorophore and an  $\alpha$ -aminoanthraquinone quencher.

6. Exhibit B of the April 12, 2010 Declaration describes various probes labeled on either the 3' or 5' end with Fluorescein (abbreviated as Fam), and labeled on the opposite end with various quenchers, including the quencher IB3.1. IB3.1, which is an abbreviation for "Iowa Black 3.1," is an  $\alpha$ -aminoanthraquinone quencher having the chemical composition shown below. See Exhibit A, which is a Preliminary Invention/Improvement Disclosure prepared by Dr. Laikhter prior to May 6, 2002 (dates redacted).



**Iowa Black 3.1**

$\lambda_{\text{max}}$  650nm

This same compound was described in Exhibit A of the April 12, 2010 Declaration at pages 25, 31 and 43 of Laboratory Notebook 325 of Dr. Laikhter.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section

101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 24, 2010

Mark Behl

Docket No. 013670-9004-US00

Q:\CLIENT\013670\9004\B2341438.1



## Preliminary Invention/Improvement Disclosure

Title: "Iowa Black 3.1" - Universal Fluorescence Quenchers for use in dual labeled DNA/RNA based probes.

Guidelines for Title: *It should not just be a trade or pet name. Rather it should include a descriptive phrase. There is no requirement to give the invention a pet name, but if writings or drawing will reference the invention using such a name, be sure to include it.*

Sample Title: "'Lighthouse'": An RNase detection system utilizing RNase H cleavage and FRET based quenching.

Date of this Disclosure: [Redacted]  
Redacted

Date of Initial Invention [Redacted]  
Redacted

Names of Other Inventors, if any.: 1. Yaw Fui Yong.

2. Mark Behlke.

3. Scott Rose.

4. Lingyan Huang.

### General Description of the Invention (include drawings if any):

This preliminary invention disclosure describes an idea of using universal fluorescence quencher in new dual labeled probes, such as molecular beacons, "RNase Alert", "DNase Alert", and probes useful for Real Time PCR reaction with wide range of fluorophores.

Several Molecular Beacons and TaqMan Probes have been synthesized using anthraquinone based quencher - Iowa Black 3, which have been described in previous invention disclosure (Invention disclosure [Redacted]).

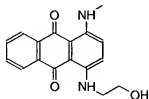
Redacted

An improved Iowa Black 3.1 quencher have been used for the synthesis of Molecular Beacon with 6-FAM fluorophore on 3' end of DNA molecule and quencher on 5'. The result is shown in Table below.

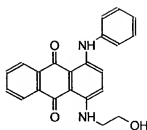
Signal to Noise of Molecular Beacon Employing Iowa Black 3.1 as a Quencher.

Quencher/Fluorophore	RP HPLC	RP + IE HPLC
IB 3.1/FAM	49	149

Chemical Structures of Iowa Black 3, and Iowa Black 3.1 are shown below.



**Iowa Black 3**  
 $\lambda_{\text{max}}$  640nm



**Iowa Black 3.1**  
 $\lambda_{\text{max}}$  650nm

Additional description attached. (if yes, then indicate number of attached pages: \_\_\_\_\_).

Corresponding Lab Notebook Numbers, if any # 325 pp. 25-26, # 334 pp. 51-52. If not applicable.

Signed \_\_\_\_\_

(Andrei Laikhter)

Date: \_\_\_\_\_

Redacted

Disclose Signed Original to Legal Department for further processing.

Redacted

EXHIBIT A

ID2001-6